16.1 | Regulation of Gene Expression

By the end of this section, you will be able to do the following:

- Discuss why every cell does not express all of its genes all of the time
- Describe how prokaryotic gene regulation occurs at the transcriptional level
- Discuss how eukaryotic gene regulation occurs at the epigenetic, transcriptional, post-transcriptional, translational, and post-translational levels

For a cell to function properly, necessary proteins must be synthesized at the proper time and place. All cells control or regulate the synthesis of proteins from information encoded in their DNA. The process of turning on a gene to produce RNA and protein is called **gene expression**. Whether in a simple unicellular organism or a complex multi-cellular organism, each cell controls when and how its genes are expressed. For this to occur, there must be internal chemical mechanisms that control when a gene is expressed to make RNA and protein, how much of the protein is made, and when it is time to stop making that protein because it is no longer needed.

The regulation of gene expression conserves energy and space. It would require a significant amount of energy for an organism to express every gene at all times, so it is more energy efficient to turn on the genes only when they are required. In addition, only expressing a subset of genes in each cell saves space because DNA must be unwound from its tightly coiled structure to transcribe and translate the DNA. Cells would have to be enormous if every protein were expressed in every cell all the time.

The control of gene expression is extremely complex. Malfunctions in this process are detrimental to the cell and can lead to the development of many diseases, including cancer.

Prokaryotic versus Eukaryotic Gene Expression

To understand how gene expression is regulated, we must first understand how a gene codes for a functional protein in a cell. The process occurs in both prokaryotic and eukaryotic cells, just in slightly different manners.

Prokaryotic organisms are single-celled organisms that lack a cell nucleus, and their DNA therefore floats freely in the cell cytoplasm. To synthesize a protein, the processes of transcription and translation occur almost simultaneously. When the resulting protein is no longer needed, transcription stops. As a result, the primary method to control what type of protein and how much of each protein is expressed in a prokaryotic cell is the regulation of DNA transcription. All of the subsequent steps occur automatically. When more protein is required, more transcription occurs. Therefore, in prokaryotic cells, the control of gene expression is mostly at the transcriptional level.

Eukaryotic cells, in contrast, have intracellular organelles that add to their complexity. In eukaryotic cells, the DNA is contained inside the cell's nucleus and there it is transcribed into RNA. The newly synthesized RNA is then transported out of the nucleus into the cytoplasm, where ribosomes translate the RNA into protein. The processes of transcription and translation are *physically separated* by the nuclear membrane; transcription occurs only within the nucleus, and translation occurs only outside the nucleus in the cytoplasm. The regulation of gene expression can occur at all stages of the process (**Figure 16.2**). Regulation may occur when the DNA is uncoiled and loosened from nucleosomes to bind transcription factors (**epigenetic** level), when the RNA is transcribed (**transcriptional** level), when the RNA is processed and exported to the cytoplasm after it is transcribed (**post-transcriptional** level), when the RNA is translated into protein (**translational** level), or after the protein has been made (**post-translational** level).

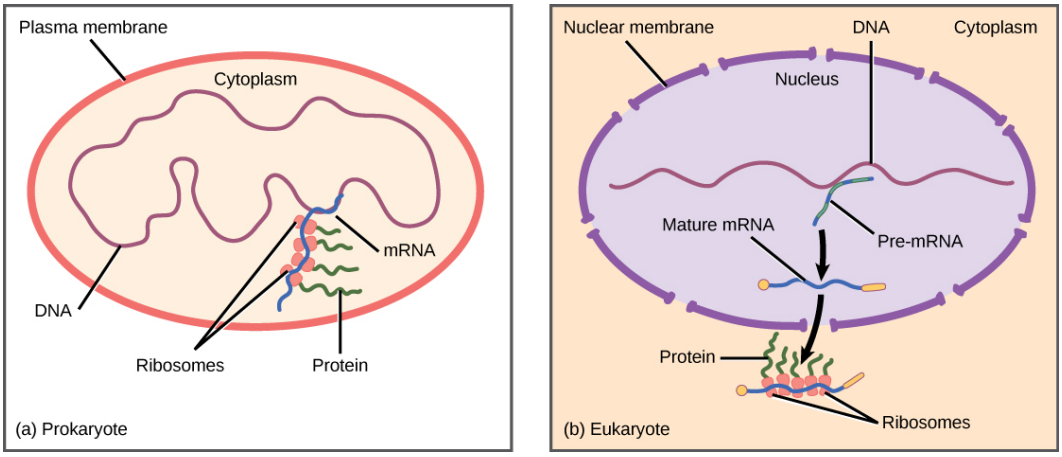

Figure 16.2 Regulation in prokaryotes and eukaryotes. Prokaryotic transcription and translation occur simultaneously in the cytoplasm, and regulation occurs at the transcriptional level. Eukaryotic gene expression is regulated during transcription and RNA processing, which take place in the nucleus, and during protein translation, which takes place in the cytoplasm. Further regulation may occur through post-translational modifications of proteins.

The differences in the regulation of gene expression between prokaryotes and eukaryotes are summarized in **Table 16.1**. The regulation of gene expression is discussed in detail in subsequent modules.

Differences in the Regulation of Gene Expression of Prokaryotic and Eukaryotic Organisms

Prokaryotic organisms	Eukaryotic organisms
Lack a membrane-bound nucleus	Contain nucleus
DNA is found in the cytoplasm	DNA is confined to the nuclear compartment
RNA transcription and protein formation occur almost simultaneously	RNA transcription occurs prior to protein formation, and it takes place in the nucleus. Translation of RNA to protein occurs in the cytoplasm.
Gene expression is regulated primarily at the transcriptional level	Gene expression is regulated at many levels (epigenetic, transcriptional, nuclear shuttling, post-transcriptional, translational, and post-translational)

Table 16.1

evolution CONNECTION

Evolution of Gene Regulation

Prokaryotic cells can only regulate gene expression by controlling the amount of transcription. As eukaryotic cells evolved, the complexity of the control of gene expression increased. For example, with the evolution of eukaryotic cells came compartmentalization of important cellular components and cellular processes. A nuclear region that contains the DNA was formed. Transcription and translation were physically separated into two different cellular compartments. It therefore became possible to control gene expression by regulating transcription in the nucleus, and also by controlling the RNA levels and protein translation present outside the nucleus.

Most gene regulation is done to conserve cell resources. However, other regulatory processes may be defensive. Cellular processes such as developed to protect the cell from viral or parasitic infections. If the cell could quickly shut off gene expression for a short period of time, it would be able to survive an infection when other organisms could not. Therefore, the organism evolved a new process that helped it survive, and it was able to pass this new development to offspring.

16.2 | Prokaryotic Gene Regulation

By the end of this section, you will be able to do the following:

- Describe the steps involved in prokaryotic gene regulation
- Explain the roles of activators, inducers, and repressors in gene regulation

The DNA of prokaryotes is organized into a circular chromosome, supercoiled within the nucleoid region of the cell cytoplasm. Proteins that are needed for a specific function, or that are involved in the same biochemical pathway, are encoded together in blocks called **operons**. For example, all of the genes needed to use lactose as an energy source are coded next to each other in the lactose (or *lac*) operon, and transcribed into a single mRNA.

In prokaryotic cells, there are three types of regulatory molecules that can affect the expression of operons: repressors, activators, and inducers. Repressors and activators are proteins produced in the cell. Both repressors and activators regulate gene expression by binding to specific DNA sites *adjacent* to the genes they control. *In general, activators bind to the promoter site, while repressors bind to operator regions*. **Repressors** prevent transcription of a gene in response to an external stimulus, whereas **activators** increase the transcription of a gene in response to an external stimulus. Inducers are small molecules that may be produced by the cell or that are in the cell's environment. Inducers either activate or repress transcription depending on the needs of the cell and the availability of substrate.

The *trp* Operon: A Repressible Operon

Bacteria such as *Escherichia coli* need amino acids to survive, and are able to synthesize many of them. **Tryptophan** is one such amino acid that *E. coli* can either ingest from the environment or synthesize using enzymes that are encoded by five genes. These five genes are next to each other in what is called the **tryptophan (*trp*) operon** (Figure 16.3). The genes are transcribed into a single mRNA, which is then translated to produce all five enzymes. If tryptophan is present in the environment, then *E. coli* does not need to synthesize it and the *trp* operon is switched off. However, when tryptophan availability is low, the switch controlling the operon is turned on, the mRNA is transcribed, the enzyme proteins are translated, and tryptophan is synthesized.

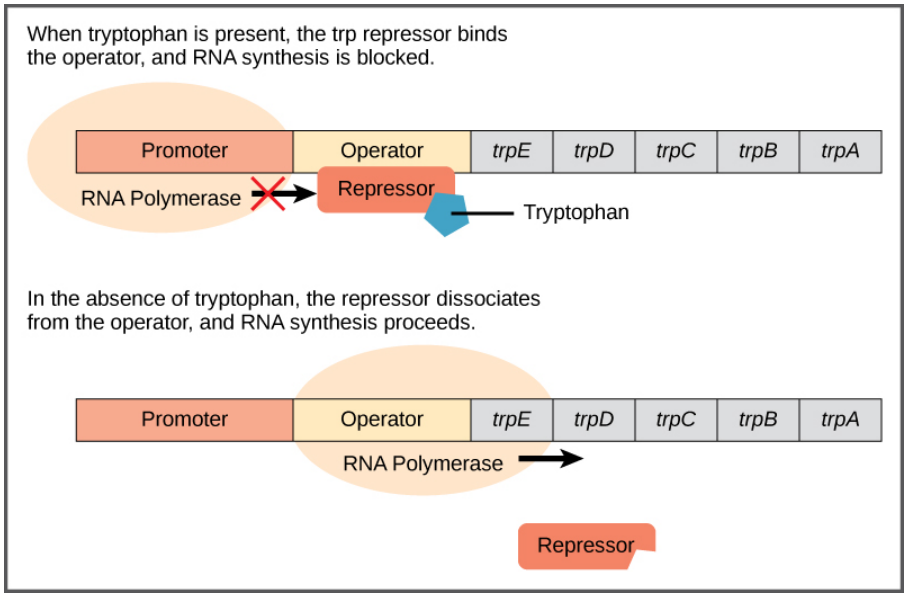

Figure 16.3 The tryptophan operon. The five genes that are needed to synthesize tryptophan in *E. coli* are located next to each other in the *trp* operon. When tryptophan is plentiful, two tryptophan molecules bind the repressor protein at the operator sequence. This physically blocks the RNA polymerase from transcribing the tryptophan genes. When tryptophan is absent, the repressor protein does not bind to the operator and the genes are transcribed.

The *trp* operon includes three important regions: the coding region, the *trp* operator and the *trp* promoter. The coding region includes the genes for the five tryptophan biosynthesis enzymes. Just before the coding region is the **transcriptional start site**. The promoter sequence, to which RNA polymerase binds to initiate transcription, is before or "upstream" of the transcriptional start site. Between the promoter and the transcriptional start site is the operator region.

The *trp* **operator** contains the DNA code to which the *trp* repressor protein can bind. However, the repressor alone cannot bind to the operator. When tryptophan is present in the cell, two tryptophan molecules bind to the *trp* repressor, which changes the shape of the repressor protein to a form that can bind to the *trp* operator. Binding of the tryptophan–repressor complex at the operator physically prevents the RNA polymerase from binding to the promoter and transcribing the downstream genes.

When tryptophan is not present in the cell, the repressor by itself does not bind to the operator, the polymerase can transcribe the enzyme genes, and tryptophan is synthesized. Because the repressor protein actively binds to the operator to keep the genes turned off, the *trp* operon is said to be *negatively regulated* and the proteins that bind to the operator to silence *trp* expression are **negative regulators**.

LINK TO LEARNING

Watch this video to learn more about the *trp* operon. **(This multimedia resource will open in a browser.) (http://cnx.org/content/m66504/1.3/#eip-id1169842033659)**

Catabolite Activator Protein (CAP): A Transcriptional Activator

Just as the *trp* operon is negatively regulated by tryptophan molecules, there are proteins that bind to the promoter sequences that act as **positive regulators** to turn genes on and activate them. For example, when glucose is scarce, *E. coli* bacteria can turn to other sugar sources for fuel. To do this, new genes to process these alternate sugars must be transcribed. When glucose levels drop, cyclic AMP (cAMP) begins to accumulate in the cell. The cAMP molecule is a signaling molecule that is involved in glucose and energy metabolism in *E. coli*. Accumulating cAMP binds to the positive regulator **catabolite activator protein (CAP)**, a protein that

binds to the promoters of operons which control the processing of alternative sugars. When cAMP binds to CAP, the complex then binds to the promoter region of the genes that are needed to use the alternate sugar sources (Figure 16.4). In these operons, a CAP-binding site is located upstream of the RNA-polymerase-binding site in the promoter. CAP binding stabilizes the binding of RNA polymerase to the promoter region and increases transcription of the associated protein-coding genes.

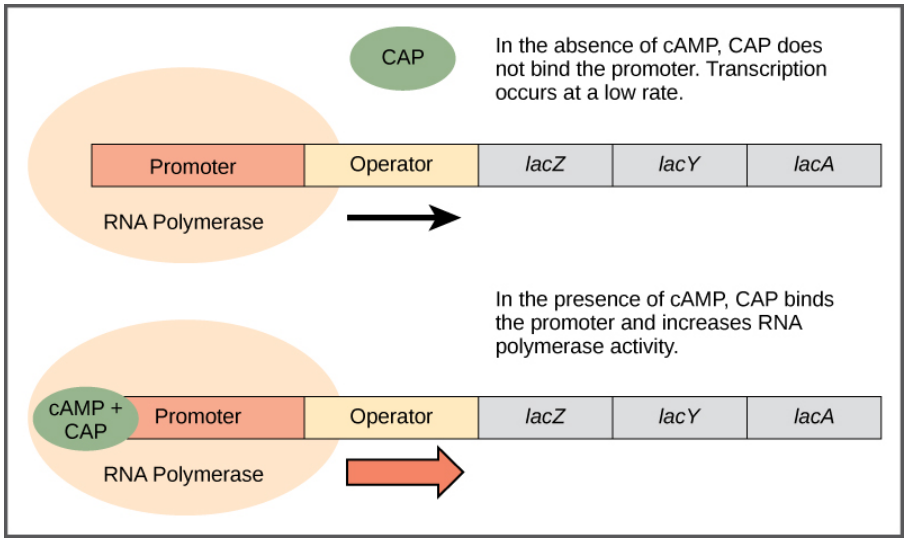

Figure 16.4 Transcriptional activation by the CAP protein. When glucose levels fall, *E. coli* may use other sugars for fuel but must transcribe new genes to do so. As glucose supplies become limited, cAMP levels increase. This cAMP binds to the CAP protein, a positive regulator that binds to a promoter region upstream of the genes required to use other sugar sources.

The *lac* Operon: An Inducible Operon

The third type of gene regulation in prokaryotic cells occurs through *inducible operons*, which have proteins that bind to activate or repress transcription depending on the local environment and the needs of the cell. The *lac* operon is a typical inducible operon. As mentioned previously, *E. coli* is able to use other sugars as energy sources when glucose concentrations are low. One such sugar source is lactose. The ***lac* operon** encodes the genes necessary to acquire and process the lactose from the local environment. The Z gene of the *lac* operon encodes beta-galactosidase, which breaks lactose down to glucose and galactose.

However, for the *lac* operon to be activated, two conditions must be met. First, the level of glucose must be very low or non-existent. Second, lactose must be present. Only when glucose is absent and lactose is present will the *lac* operon be transcribed (Figure 16.5). In the absence of glucose, the binding of the CAP protein makes transcription of the *lac* operon more effective. When lactose is present, it binds to the *lac* repressor and changes its shape so that it cannot bind to the *lac* operator to prevent transcription. This combination of conditions makes sense for the cell, because it would be energetically wasteful to synthesize the enzymes to process lactose if glucose was plentiful or lactose was not available.

visual CONNECTION

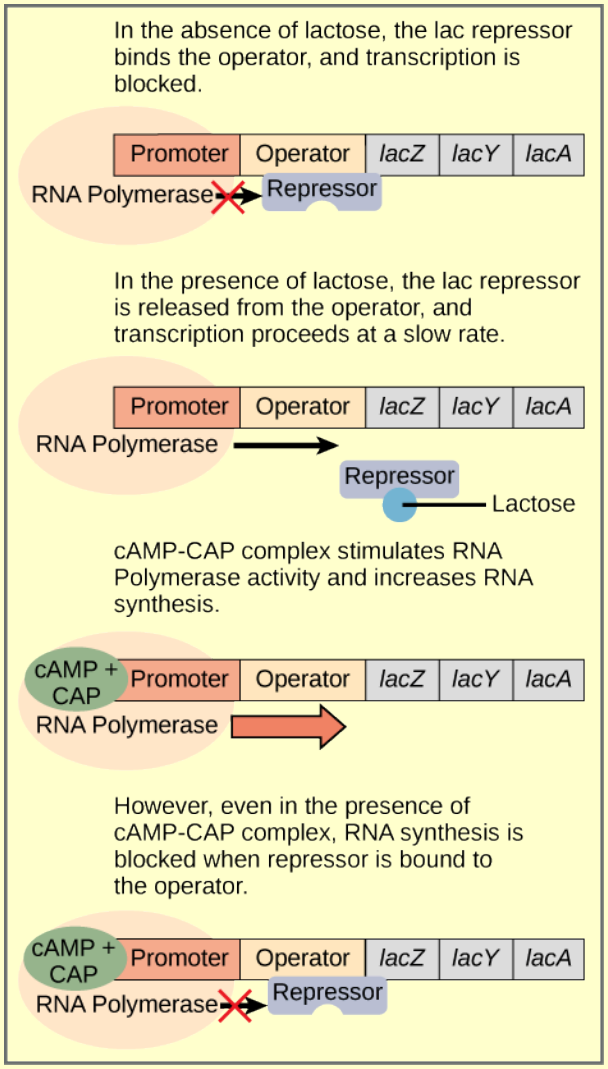

Figure 16.5 Regulation of the *lac* operon. Transcription of the *lac* operon is carefully regulated so that its expression only occurs when glucose is limited and lactose is present to serve as an alternative fuel source.

Question: In *E. coli*, the *trp* operon is on by default, while the *lac* operon is off. Why do you think this is the case?

If glucose is present, then CAP fails to bind to the promoter sequence to activate transcription. If lactose is absent, then the repressor binds to the operator to prevent transcription. If either of these conditions is met, then transcription remains off. Only when glucose is absent and lactose is present is the *lac* operon transcribed (Table 16.2).

Signals that Induce or Repress Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	-	-	+	No

Table 16.2

Signals that Induce or Repress Transcription of the *lac* Operon

Glucose	CAP binds	Lactose	Repressor binds	Transcription
+	-	+	-	Some
-	+	-	+	No
-	+	+	-	Yes

Table 16.2

Watch an animated tutorial about the workings of *lac* operon here. **(This multimedia resource will open in a browser.) (http://cnx.org/content/m66504/1.3/#eip-id1165239273914)**

16.3 | Eukaryotic Epigenetic Gene Regulation

By the end of this section, you will be able to do the following:

- Explain how chromatin remodeling controls transcriptional access
- Describe how access to DNA is controlled by histone modification
- Describe how DNA methylation is related to epigenetic gene changes

Eukaryotic gene expression is more complex than prokaryotic gene expression because the processes of transcription and translation are physically separated. Unlike prokaryotic cells, eukaryotic cells can regulate gene expression at many different levels. Epigenetic changes are inheritable changes in gene expression that do not result from changes in the DNA sequence. Eukaryotic gene expression begins with control of access to the DNA. Transcriptional access to the DNA can be controlled in two general ways: chromatin remodeling and DNA methylation. Chromatin remodeling changes the way that DNA is associated with chromosomal histones. DNA methylation is associated with developmental changes and gene silencing.

Epigenetic Control: Regulating Access to Genes within the Chromosome

The human genome encodes over 20,000 genes, with hundreds to thousands of genes on each of the 23 human chromosomes. The DNA in the nucleus is precisely wound, folded, and compacted into chromosomes so that it will fit into the nucleus. It is also organized so that specific segments can be accessed as needed by a specific cell type.

The first level of organization, or **packing**, is the winding of DNA strands around histone proteins. Histones package and order DNA into structural units called nucleosome complexes, which can control the access of proteins to the DNA regions (**Figure 16.6a**). Under the electron microscope, this winding of DNA around histone proteins to form nucleosomes looks like small beads on a string (**Figure 16.6b**).

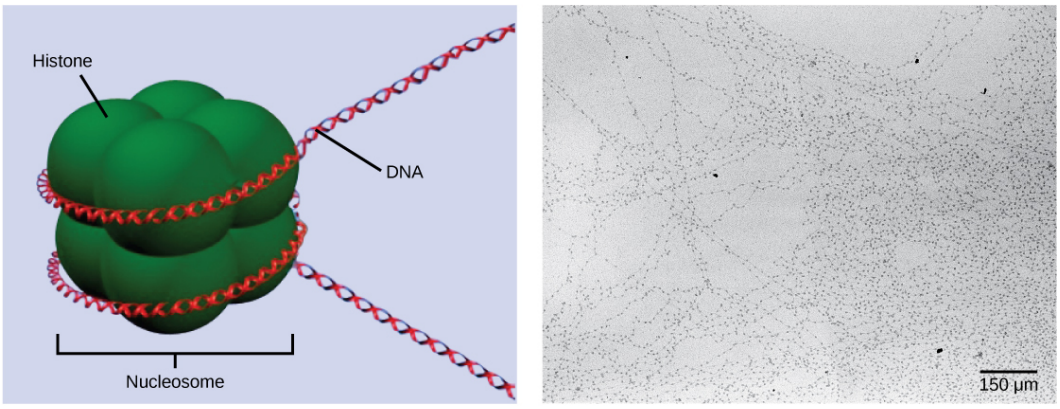

Figure 16.6 DNA is folded around histone proteins to create (a) nucleosome complexes. These nucleosomes control the access of proteins to the underlying DNA. When viewed through an electron microscope (b), the nucleosomes look like beads on a string. (credit “micrograph”: modification of work by Chris Woodcock)

These beads (histone proteins) can move along the string (DNA) to expose different sections of the molecule. If DNA encoding a specific gene is to be transcribed into RNA, the nucleosomes surrounding that region of DNA can slide down the DNA to open that specific chromosomal region and allow for the transcriptional machinery (RNA polymerase) to initiate transcription (**Figure 16.7**).

visual CONNECTION

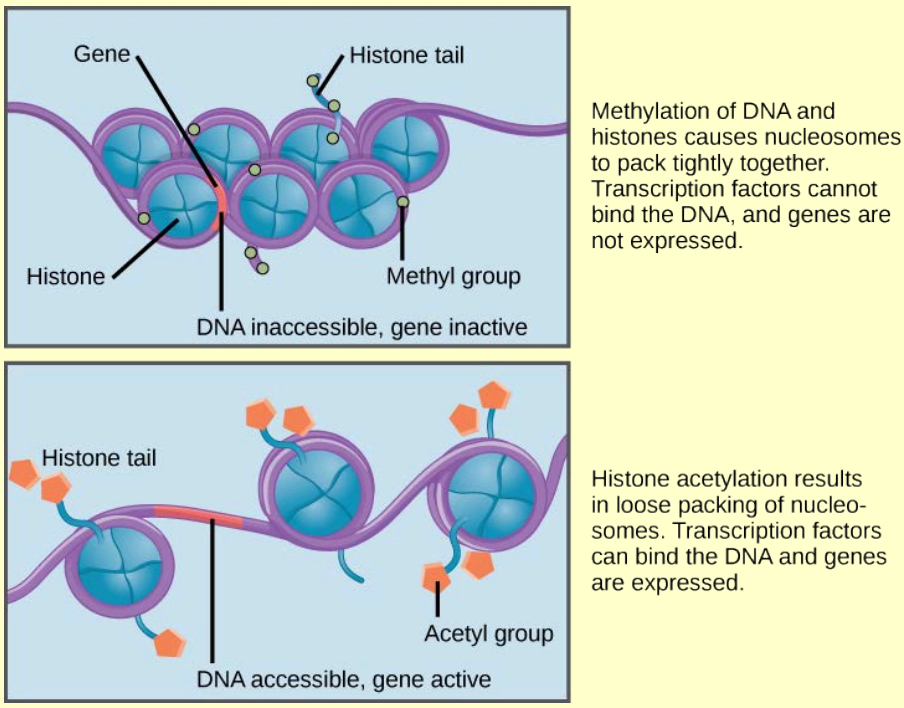

Figure 16.7 Nucleosomes can slide along DNA. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the nucleosomes are spaced far apart (bottom), the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. Modifications to the histones and DNA affect nucleosome spacing.

In females, one of the two X chromosomes is inactivated during embryonic development because of epigenetic changes to the chromatin. What impact do you think these changes would have on nucleosome packing?

How closely the histone proteins associate with the DNA is regulated by signals found on both the histone proteins and on the DNA. These signals are functional groups added to histone proteins or to DNA and determine whether a chromosomal region should be open or closed (**Figure 16.8** depicts modifications to histone proteins and DNA). These tags are not permanent, but may be added or removed as needed. Some chemical groups (phosphate, methyl, or acetyl groups) are attached to specific amino acids in histone "tails" at the N-terminus of the protein. These groups do not alter the DNA base sequence, but they do alter how tightly wound the DNA is around the histone proteins. DNA is a negatively charged molecule and unmodified histones are positively charged; therefore, changes in the charge of the histone will change how tightly wound the DNA molecule will be. By adding chemical modifications like acetyl groups, the charge becomes less positive, and the binding of DNA to the histones is relaxed. Altering the location of nucleosomes and the tightness of histone binding opens some regions of chromatin to transcription and closes others.

The DNA molecule itself can also be modified by methylation. DNA methylation occurs within very specific regions called CpG islands. These are stretches with a high frequency of cytosine and guanine dinucleotide DNA pairs (CG) found in the promoter regions of genes. The cytosine member of the CG pair can be methylated (a methyl group is added). Methylated genes are usually silenced, although methylation may have other regulatory effects. In some cases, genes that are silenced during the development of the gametes of one parent are transmitted in their silenced condition to the offspring. Such genes are said to be imprinted. Parental diet or other environmental conditions may also affect the methylation patterns of genes, which in turn modifies gene expression. Changes in chromatin organization interact with DNA methylation. DNA methyltransferases appear to be attracted to chromatin regions with specific histone modifications. Highly methylated (*hypermethylated*) DNA regions with deacetylated histones are tightly coiled and transcriptionally inactive.

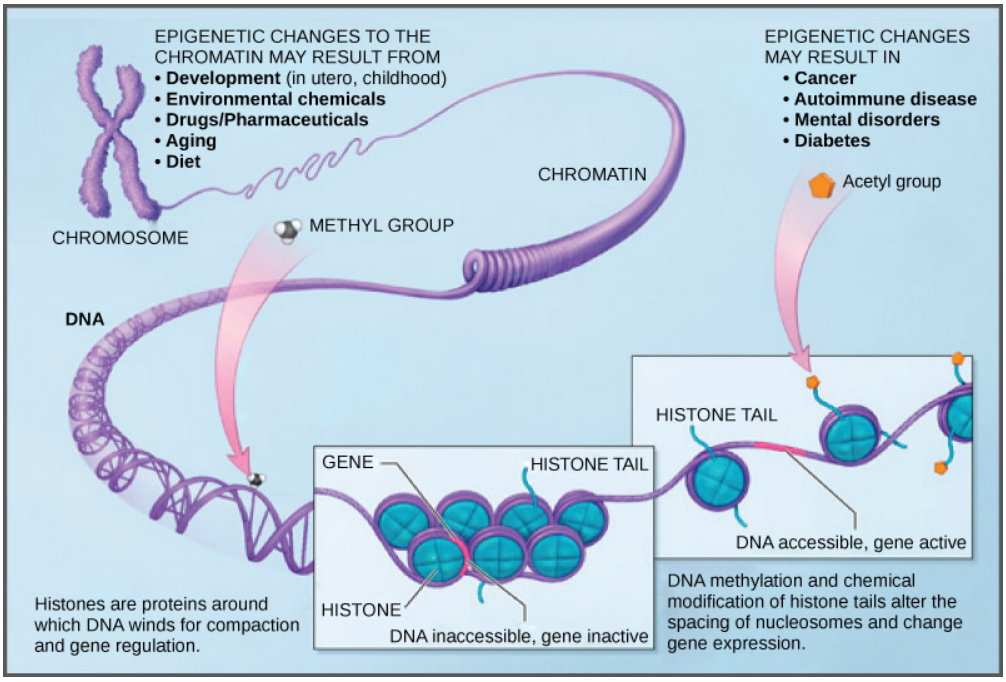

Figure 16.8 Histone proteins and DNA nucleotides can be modified chemically. Modifications affect nucleosome spacing and gene expression. (credit: modification of work by NIH)

Epigenetic changes are not permanent, although they often persist through multiple rounds of cell division and may even cross generational lines. Chromatin remodeling alters the chromosomal structure (open or closed) as needed. If a gene is to be transcribed, the histone proteins and DNA in the chromosomal region encoding that gene are modified in a way that opens the promoter region to allow RNA polymerase and other proteins, called **transcription factors**, to bind and initiate transcription. If a gene is to remain turned off, or silenced, the histone proteins and DNA have different modifications that signal a closed chromosomal configuration. In this closed configuration, the RNA polymerase and transcription factors do not have access to the DNA and transcription cannot occur (**Figure 16.8**).

View this video that describes how epigenetic regulation controls gene expression. **(This multimedia resource will open in a browser.) (http://cnx.org/content/m66505/1.3/#eip-id1169842033590)**

16.4 | Eukaryotic Transcription Gene Regulation

By the end of this section, you will be able to do the following:

- Discuss the role of transcription factors in gene regulation
- Explain how enhancers and repressors regulate gene expression

Like prokaryotic cells, the transcription of genes in eukaryotes requires the action of an RNA polymerase to bind to a DNA sequence upstream of a gene in order to initiate transcription. However, unlike prokaryotic cells, the eukaryotic RNA polymerase requires other proteins, or transcription factors, to facilitate transcription initiation. RNA polymerase by itself cannot initiate transcription in eukaryotic cells. There are two types of transcription factors that regulate eukaryotic transcription: *General (or basal) transcription factors* bind to the core promoter region to assist with the binding of RNA polymerase. *Specific transcription factors* bind to various regions outside of the core promoter region and interact with the proteins at the core promoter to enhance or repress the activity of the polymerase.

View the process of transcription—the making of RNA from a DNA template. **(This multimedia resource will open in a browser.) (http://cnx.org/content/m66506/1.3/#eip-id1168020166468)**

The Promoter and the Transcription Machinery

Genes are organized to make the control of gene expression easier. The promoter region is immediately upstream of the coding sequence. This region can be short (only a few nucleotides in length) or quite long (hundreds of nucleotides long). The longer the promoter, the more available space for proteins to bind. This also adds more control to the transcription process. The length of the promoter is gene-specific and can differ dramatically between genes. Consequently, the level of control of gene expression can also differ quite dramatically between genes. The purpose of the **promoter** is to bind transcription factors that control the initiation of transcription.

Within the core promoter region, 25 to 35 bases upstream of the transcriptional start site, resides the TATA box. The TATA box has the consensus sequence of 5'-TATAAA-3'. The TATA box is the binding site for a protein complex called TFIID, which contains a TATA-binding protein. Binding of TFIID recruits other transcription factors, including TFIIB, TFIIE, TFIIF, and TFIIH. Some of these transcription factors help to bind the RNA polymerase to the promoter, and others help to activate the transcription initiation complex.

In addition to the TATA box, other binding sites are found in some promoters. Some biologists prefer to restrict the range of the eukaryotic promoter to the core promoter, or polymerase binding site, and refer to these

additional sites as promoter-proximal elements, because they are usually found within a few hundred base pairs upstream of the transcriptional start site. Examples of these elements are the CAAT box, with the consensus sequence 5'-CCAAT-3' and the GC box, with the consensus sequence 5'-GGGCGG-3'. Specific transcription factors can bind to these promoter-proximal elements to regulate gene transcription. A given gene may have its own combination of these specific transcription-factor binding sites. There are hundreds of transcription factors in a cell, each of which binds specifically to a particular DNA sequence motif. When transcription factors bind to the promoter just upstream of the encoded gene, it is referred to as a **cis-acting element**, because it is on the same chromosome just next to the gene. Transcription factors respond to environmental stimuli that cause the proteins to find their binding sites and initiate transcription of the gene that is needed.

Enhancers and Transcription

In some eukaryotic genes, there are additional regions that help increase or enhance transcription. These regions, called **enhancers**, are not necessarily close to the genes they enhance. They can be located upstream of a gene, within the coding region of the gene, downstream of a gene, or may be thousands of nucleotides away.

Enhancer regions are binding sequences, or sites, for specific transcription factors. When a protein transcription factor binds to its enhancer sequence, the shape of the protein changes, allowing it to interact with proteins at the promotor site. However, since the enhancer region may be distant from the promoter, the DNA must bend to allow the proteins at the two sites to come into contact. DNA bending proteins help to bend the DNA and bring the enhancer and promoter regions together (Figure 16.9). This shape change allows for the interaction of the specific activator proteins bound to the enhancers with the general transcription factors bound to the promoter region and the RNA polymerase.

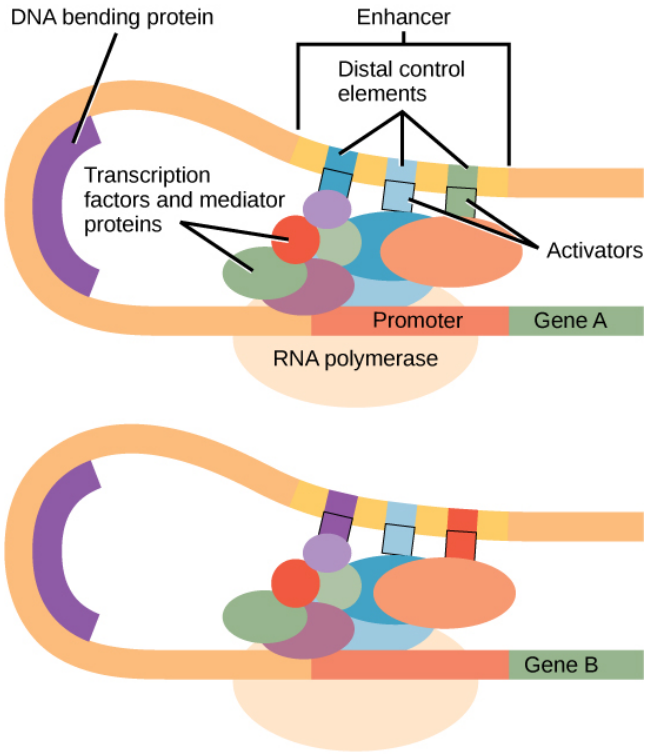

Figure 16.9 Interaction between proteins at the promoter and enhancer sites. An enhancer is a DNA sequence that promotes transcription. Each enhancer is made up of short DNA sequences called distal control elements. Activators bound to the distal control elements interact with mediator proteins and transcription factors. Two different genes may have the same promoter but different distal control elements, enabling differential gene expression.

Turning Genes Off: Transcriptional Repressors

Like prokaryotic cells, eukaryotic cells also have mechanisms to prevent transcription. Transcriptional repressors can bind to promoter or enhancer regions and block transcription. Like the transcriptional activators, repressors respond to external stimuli to prevent the binding of activating transcription factors.

16.5 | Eukaryotic Post-transcriptional Gene Regulation

By the end of this section, you will be able to do the following:

- Understand RNA splicing and explain its role in regulating gene expression
- Describe the importance of RNA stability in gene regulation

RNA is transcribed, but must be processed into a mature form before translation can begin. This processing that takes place after an RNA molecule has been transcribed, but before it is translated into a protein, is called *post-transcriptional modification*. As with the epigenetic and transcriptional stages of processing, this post-transcriptional step can also be regulated to control gene expression in the cell. If the RNA is not processed, shuttled, or translated, then no protein will be synthesized.

RNA Splicing, the First Stage of Post-transcriptional Control

In eukaryotic cells, the RNA transcript often contains regions, called introns, that are removed prior to translation. The regions of RNA that code for protein are called **exons**. (Figure 16.10). After an RNA molecule has been transcribed, but prior to its departure from the nucleus to be translated, the RNA is processed and the introns are removed by splicing. Splicing is done by spliceosomes, ribonucleoprotein complexes that can recognize the two ends of the intron, cut the transcript at those two points, and bring the exons together for ligation.

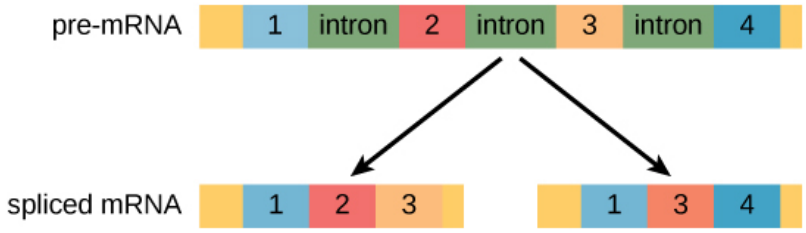

Figure 16.10 Pre-mRNA can be alternatively spliced to create different proteins.

evolution CONNECTION

Alternative RNA Splicing

In the 1970s, genes were first observed that exhibited alternative RNA splicing. Alternative RNA splicing is a mechanism that allows different protein products to be produced from one gene when different combinations of exons are combined to form the mRNA (Figure 16.11). This alternative splicing can be haphazard, but more often it is controlled and acts as a mechanism of *gene regulation*, with the frequency of different splicing alternatives controlled by the cell as a way to control the production of different protein products in different cells or at different stages of development. Alternative splicing is now understood to be a common mechanism of gene regulation in eukaryotes; according to one estimate, 70 percent of genes in humans are expressed as multiple proteins through alternative splicing. Although there are multiple ways to alternatively splice RNA transcripts, the original 5'-3' order of the exons is *always conserved*. That is, a transcript with exons 1 2 3 4 5 6 7 might be spliced 1 2 4 5 6 7 or 1 2 3 6 7, but never 1 2 5 4 3 6 7.

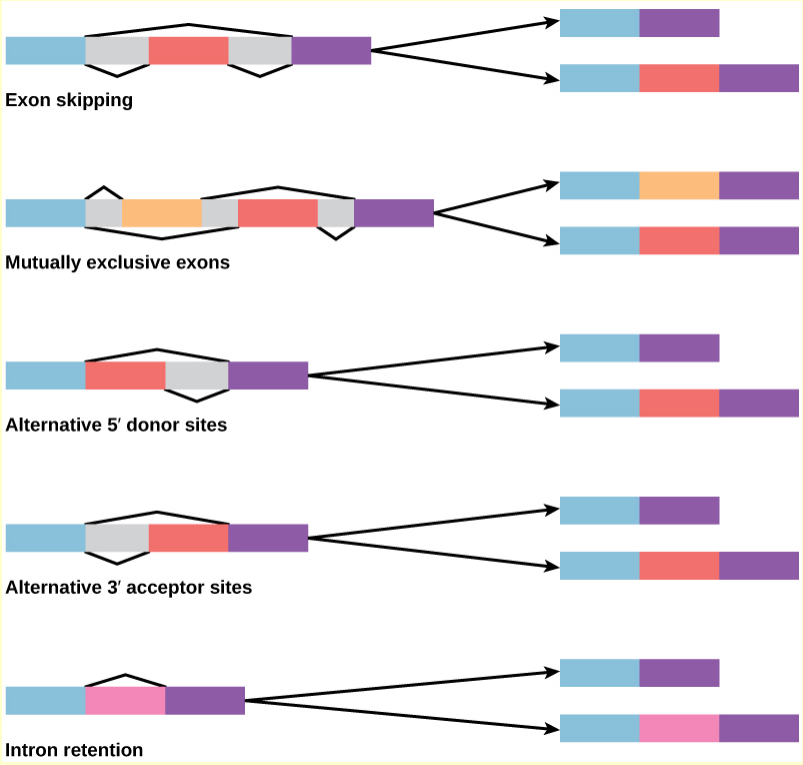

Figure 16.11 There are five basic modes of alternative splicing.

evolution CONNECTION

How could alternative splicing evolve? Introns have a beginning- and ending-recognition sequence; it is easy to imagine the failure of the splicing mechanism to identify the end of an intron and instead find the end of the next intron, thus removing two introns and the intervening exon. In fact, there are mechanisms in place to prevent such intron skipping, but mutations are likely to lead to their failure. Such “mistakes” would more than likely produce a nonfunctional protein. Indeed, the cause of many genetic diseases is abnormal splicing rather than mutations in a coding sequence. However, alternative splicing could possibly create a protein variant without the loss of the original protein, opening up possibilities for adaptation of the new variant to new functions. Gene duplication has played an important role in the evolution of new functions in a similar way by providing genes that may evolve without eliminating the original, functional protein.

Question: In the corn snake *Pantherophis guttatus*, there are several different color variants, including amelanistic snakes whose skin patterns display only red and yellow pigments. The cause of amelanism in these snakes was recently identified as the insertion of a transposable element into an intron in the OCA2 (oculocutaneous albinism) gene. How might the insertion of extra genetic material into an intron lead to a nonfunctional protein?

LINK TO LEARNING

Visualize how mRNA splicing happens by watching the process in action in this video. **(This multimedia resource will open in a browser.) (http://cnx.org/content/m66507/1.5/#eip-id1171119155972)**

Control of RNA Stability

Before the mRNA leaves the nucleus, it is given two protective "caps" that prevent the ends of the strand from degrading during its journey. 5' and 3' exonucleases can degrade unprotected RNAs. The **5' cap**, which is placed on the 5' end of the mRNA, is usually composed of a methylated guanosine triphosphate molecule (GTP). The GTP is placed "backward" on the 5' end of the mRNA, so that the 5' carbons of the GTP and the terminal nucleotide are linked through three phosphates. The **poly-A tail**, which is attached to the 3' end, is usually composed of a long chain of adenine nucleotides. These changes protect the two ends of the RNA from exonuclease attack.

Once the RNA is transported to the cytoplasm, the length of time that the RNA resides there can be controlled. Each RNA molecule has a defined lifespan and decays at a specific rate. This rate of decay can influence how much protein is in the cell. If the decay rate is increased, the RNA will not exist in the cytoplasm as long, shortening the time available for translation of the mRNA to occur. Conversely, if the rate of decay is decreased, the mRNA molecule will reside in the cytoplasm longer and more protein can be translated. This rate of decay is referred to as the RNA stability. If the RNA is stable, it will be detected for longer periods of time in the cytoplasm.

Binding of proteins to the RNA can also influence its stability. Proteins called **RNA-binding proteins**, or RBPs, can bind to the regions of the mRNA just upstream or downstream of the protein-coding region. These regions in the RNA that are not translated into protein are called the **untranslated regions**, or UTRs. They are not introns (those have been removed in the nucleus). Rather, these are regions that regulate mRNA localization, stability, and protein translation. The region just before the protein-coding region is called the **5' UTR**, whereas the region after the coding region is called the **3' UTR** (Figure 16.12). The binding of RBPs to these regions can increase or decrease the stability of an RNA molecule, depending on the specific RBP that binds.

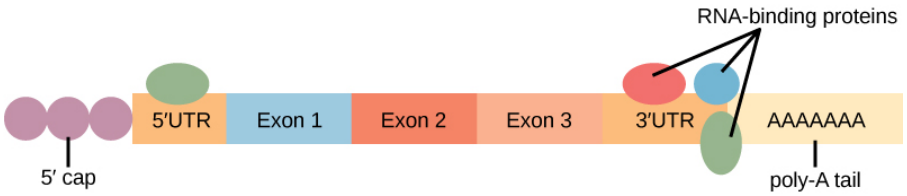

Figure 16.12 RNA-binding proteins. The protein-coding region of this processed mRNA is flanked by 5' and 3' untranslated regions (UTRs). The presence of RNA-binding proteins at the 5' or 3' UTR influences the stability of the RNA molecule.

RNA Stability and microRNAs

In addition to RBPs that bind to and control (increase or decrease) RNA stability, other elements called microRNAs can bind to the RNA molecule. These **microRNAs**, or miRNAs, are short RNA molecules that are only 21 to 24 nucleotides in length. The miRNAs are made in the nucleus as longer pre-miRNAs. These pre-miRNAs are chopped into mature miRNAs by a protein called ***Dicer***. Like transcription factors and RBPs, mature miRNAs recognize a specific sequence and bind to the RNA; however, miRNAs also associate with a ribonucleoprotein complex called the **RNA-induced silencing complex (RISC)**. The RNA component of the RISC base-pairs with complementary sequences on an mRNA and either impede translation of the message or lead to the degradation of the mRNA.

16.6 | Eukaryotic Translational and Post-translational Gene Regulation

By the end of this section, you will be able to do the following:

- Understand the process of translation and discuss its key factors
- Describe how the initiation complex controls translation
- Explain the different ways in which the post-translational control of gene expression takes place

After RNA has been transported to the cytoplasm, it is translated into protein. Control of this process is largely dependent on the RNA molecule. As previously discussed, the stability of the RNA will have a large impact on its translation into a protein. As the stability changes, the amount of time that it is available for translation also changes.

The Initiation Complex and Translation Rate

Like transcription, translation is controlled by proteins that bind and initiate the process. In translation, the complex that assembles to start the process is referred to as the translation **initiation complex**. In eukaryotes, translation is initiated by binding the initiating met-tRNAi to the 40S ribosome. This tRNA is brought to the 40S ribosome by a protein initiation factor, **eukaryotic initiation factor-2 (eIF-2)**. The eIF-2 protein binds to the high-energy molecule **guanosine triphosphate (GTP)**. The tRNA-eIF2-GTP complex then binds to the 40S ribosome. A second complex forms on the mRNA. Several different initiation factors recognize the 5' cap of the mRNA and proteins bound to the poly-A tail of the same mRNA, forming the mRNA into a loop. The cap-binding protein eIF4F brings the mRNA complex together with the 40S ribosome complex. The ribosome then scans along the mRNA until it finds a start codon AUG. When the anticodon of the initiator tRNA and the start codon are aligned, the GTP is hydrolyzed, the initiation factors are released, and the large **60S ribosomal subunit** binds to form the translation complex. The binding of eIF-2 to the RNA is controlled by phosphorylation. If eIF-2 is phosphorylated, it undergoes a conformational change and cannot bind to GTP. Therefore, the initiation complex cannot form properly and translation is impeded (Figure 16.13). When eIF-2 remains unphosphorylated, the initiation complex can form normally and translation can proceed.

visual CONNECTION

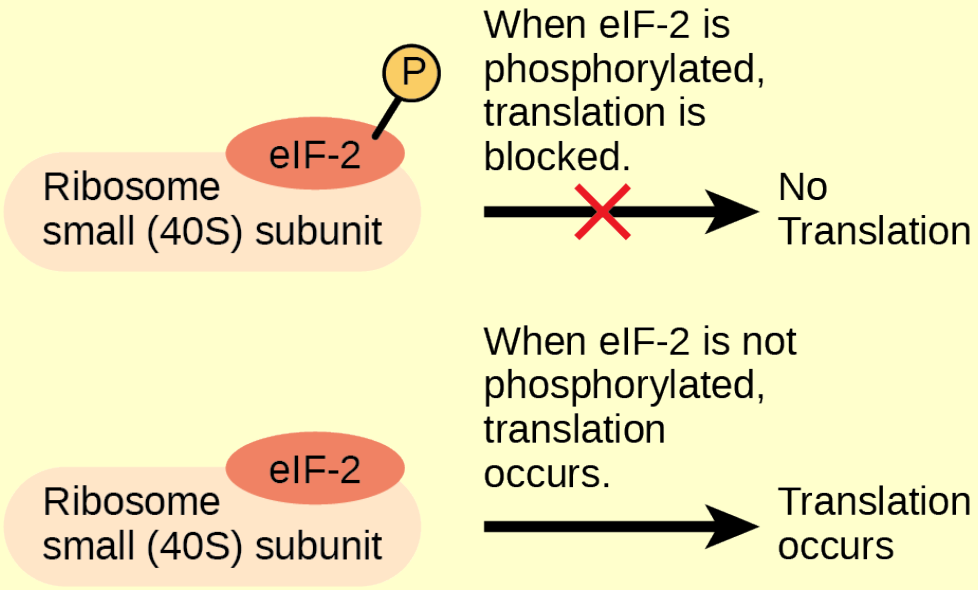

Figure 16.13 Gene expression can be controlled by factors that bind the translation initiation complex.

An increase in phosphorylation levels of eIF-2 has been observed in patients with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's. What impact do you think this might have on protein synthesis?

Chemical Modifications, Protein Activity, and Longevity

Proteins can be chemically modified with the addition of groups including methyl, phosphate, acetyl, and ubiquitin groups. The addition or removal of these groups from proteins regulates their activity or the length of time they exist in the cell. Sometimes these modifications can regulate where a protein is found in the cell—for example, in the nucleus, in the cytoplasm, or attached to the plasma membrane.

Chemical modifications occur in response to external stimuli such as stress, the lack of nutrients, heat, or ultraviolet light exposure. These changes can alter epigenetic accessibility, transcription, mRNA stability, or translation—all resulting in changes in expression of various genes. This is an efficient way for the cell to rapidly change the levels of specific proteins in response to the environment. Because proteins are involved in every stage of gene regulation, the phosphorylation of a protein (depending on the protein that is modified) can alter accessibility to the chromosome, can alter translation (by altering transcription factor binding or function), can change nuclear shuttling (by influencing modifications to the nuclear pore complex), can alter RNA stability (by binding or not binding to the RNA to regulate its stability), can modify translation (increase or decrease), or can change post-translational modifications (add or remove phosphates or other chemical modifications).

The addition of an ubiquitin group to a protein marks that protein for degradation. Ubiquitin acts like a flag indicating that the protein lifespan is complete. These proteins are moved to the **proteasome**, an organelle that functions to remove proteins, to be degraded (**Figure 16.14**). One way to control gene expression, therefore, is to alter the longevity of the protein.

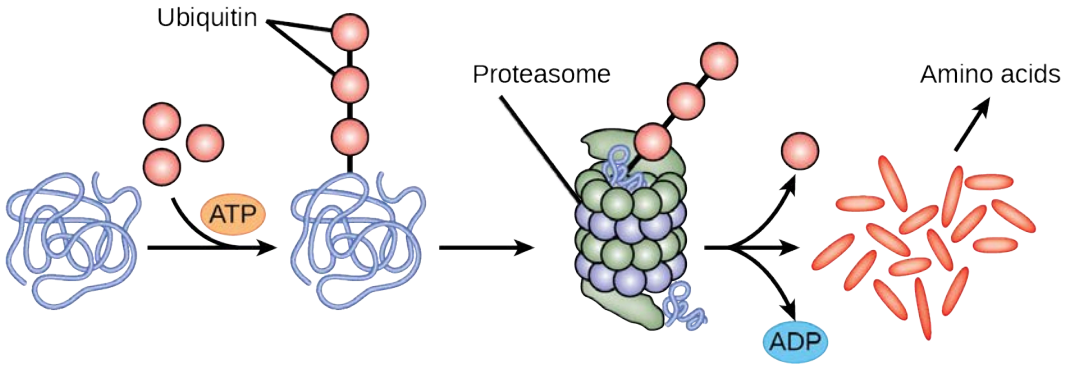

Figure 16.14 Proteins with ubiquitin tags are marked for degradation within the proteasome.

16.7 | Cancer and Gene Regulation

By the end of this section, you will be able to do the following:

- Describe how changes to gene expression can cause cancer
- Explain how changes to gene expression at different levels can disrupt the cell cycle
- Discuss how understanding regulation of gene expression can lead to better drug design

Cancer is not a single disease but includes many different diseases. In cancer cells, mutations modify cell-cycle control and cells don't stop growing as they normally would. Mutations can also alter the growth rate or the progression of the cell through the cell cycle. One example of a gene modification that alters the growth rate is increased phosphorylation of cyclin B, a protein that controls the progression of a cell through the cell cycle and serves as a cell-cycle checkpoint protein.

For cells to move through each phase of the cell cycle, the cell must pass through checkpoints. This ensures that the cell has properly completed the step and has not encountered any mutation that will alter its function. Many proteins, including cyclin B, control these checkpoints. The phosphorylation of cyclin B, a post-translational event, alters its function. As a result, cells can progress through the cell cycle unimpeded, even if mutations exist in the cell and its growth should be terminated. This post-translational change of cyclin B prevents it from controlling the cell cycle and contributes to the development of cancer.

Cancer: Disease of Altered Gene Expression

Cancer can be described as a disease of altered gene expression. There are many proteins that are turned on or off (gene activation or gene silencing) that dramatically alter the overall activity of the cell. A gene that is not normally expressed in that cell can be switched on and expressed at high levels. This can be the result of gene mutation or changes in gene regulation (epigenetic, transcription, post-transcription, translation, or post-translation).

Changes in epigenetic regulation, transcription, RNA stability, protein translation, and post-translational control can be detected in cancer. While these changes don't occur simultaneously in one cancer, changes at each of these levels can be detected when observing cancer at different sites in different individuals. Therefore, changes in **histone acetylation** (epigenetic modification that leads to gene silencing), activation of transcription factors by phosphorylation, increased RNA stability, increased translational control, and protein modification can all be detected at some point in various cancer cells. Scientists are working to understand the common changes that give rise to certain types of cancer or how a modification might be exploited to destroy a tumor cell.

Tumor Suppressor Genes, Oncogenes, and Cancer

In normal cells, some genes function to prevent excess, inappropriate cell growth. These are tumor-suppressor genes, which are active in normal cells to prevent uncontrolled cell growth. There are many tumor-suppressor genes in cells. The most studied tumor-suppressor gene is p53, which is mutated in over 50 percent of all cancer types. The p53 protein itself functions as a transcription factor. It can bind to sites in the promoters of genes to initiate transcription. Therefore, the mutation of p53 in cancer will dramatically alter the transcriptional activity of its target genes.

LINK TO LEARNING

Watch **this animation (http://openstaxcollege.org/l/p53_cancer)** to learn more about the use of p53 in fighting cancer.

Proto-oncogenes are positive cell-cycle regulators. When mutated, proto-oncogenes can become oncogenes and cause cancer. Overexpression of the oncogene can lead to uncontrolled cell growth. This is because oncogenes can alter transcriptional activity, stability, or protein translation of another gene that directly or indirectly controls cell growth. An example of an oncogene involved in cancer is a protein called myc. **Myc** is a transcription factor that is aberrantly activated in Burkett's Lymphoma, a cancer of the lymph system. Overexpression of myc transforms normal B cells into cancerous cells that continue to grow uncontrollably. High B-cell numbers can result in tumors that can interfere with normal bodily function. Patients with Burkett's lymphoma can develop tumors on their jaw or in their mouth that interfere with the ability to eat.

Cancer and Epigenetic Alterations

Silencing genes through epigenetic mechanisms is also very common in cancer cells. There are characteristic modifications to histone proteins and DNA that are associated with silenced genes. In cancer cells, the DNA in the promoter region of silenced genes is methylated on cytosine DNA residues in CpG islands. Histone proteins that surround that region lack the acetylation modification that is present when the genes are expressed in normal cells. This combination of DNA methylation and histone deacetylation (epigenetic modifications that lead to gene silencing) is commonly found in cancer. When these modifications occur, the gene present in that chromosomal region is silenced. Increasingly, scientists understand how epigenetic changes are altered in cancer. Because these changes are temporary and can be reversed—for example, by preventing the action of the histone deacetylase protein that removes acetyl groups, or by DNA methyl transferase enzymes that add methyl groups to cytosines in DNA—it is possible to design new drugs and new therapies to take advantage of the reversible nature of these processes. Indeed, many researchers are testing how a silenced gene can be switched back on in a cancer cell to help re-establish normal growth patterns.

Genes involved in the development of many other illnesses, ranging from allergies to inflammation to autism, are thought to be regulated by epigenetic mechanisms. As our knowledge of how genes are controlled deepens, new ways to treat diseases like cancer will emerge.

Cancer and Transcriptional Control

Alterations in cells that give rise to cancer can affect the transcriptional control of gene expression. Mutations that activate transcription factors, such as increased phosphorylation, can increase the binding of a transcription factor to its binding site in a promoter. This could lead to increased transcriptional activation of that gene that results in modified cell growth. Alternatively, a mutation in the DNA of a promoter or enhancer region can increase the binding ability of a transcription factor. This could also lead to the increased transcription and aberrant gene expression that is seen in cancer cells.

Researchers have been investigating how to control the transcriptional activation of gene expression in cancer. Identifying how a transcription factor binds, or a pathway that activates where a gene can be turned off, has led to new drugs and new ways to treat cancer. In breast cancer, for example, many proteins are overexpressed. This can lead to increased phosphorylation of key transcription factors that increase transcription. One such example is the overexpression of the epidermal growth-factor receptor (EGFR) in a subset of breast cancers. The EGFR pathway activates many protein kinases that, in turn, activate many transcription factors which control genes involved in cell growth. New drugs that prevent the activation of EGFR have been developed and are used to treat these cancers.

Cancer and Post-transcriptional Control

Changes in the post-transcriptional control of a gene can also result in cancer. Recently, several groups of researchers have shown that specific cancers have altered expression of miRNAs. Because miRNAs bind to the 3' UTR of RNA molecules to degrade them, overexpression of these miRNAs could be detrimental to normal

cellular activity. Too many miRNAs could dramatically decrease the RNA population, leading to a decrease in protein expression. Several studies have demonstrated a change in the miRNA population in specific cancer types. It appears that the subset of miRNAs expressed in breast cancer cells is quite different from the subset expressed in lung cancer cells or even from normal breast cells. This suggests that alterations in miRNA activity can contribute to the growth of breast cancer cells. These types of studies also suggest that if some miRNAs are specifically expressed only in cancer cells, they could be potential drug targets. It would, therefore, be conceivable that new drugs that turn off miRNA expression in cancer could be an effective method to treat cancer.

Cancer and Translational/Post-translational Control

There are many examples of how translational or post-translational modifications of proteins arise in cancer. Modifications are found in cancer cells from the increased translation of a protein to changes in protein phosphorylation to alternative splice variants of a protein. An example of how the expression of an alternative form of a protein can have dramatically different outcomes is seen in colon cancer cells. The c-Flip protein, a protein involved in mediating the cell-death pathway, comes in two forms: long (c-FLIPL) and short (c-FLIPS). Both forms appear to be involved in initiating controlled cell-death mechanisms in normal cells. However, in colon cancer cells, expression of the long form results in increased cell growth instead of cell death. Clearly, the expression of the wrong protein dramatically alters cell function and contributes to the development of cancer.

New Drugs to Combat Cancer: Targeted Therapies

Scientists are using what is known about the regulation of gene expression in disease states, including cancer, to develop new ways to treat and prevent disease development. Many scientists are designing drugs on the basis of the gene expression patterns within individual tumors. This idea, that therapy and medicines can be tailored to an individual, has given rise to the field of personalized medicine. With an increased understanding of gene regulation and gene function, medicines can be designed to specifically target diseased cells without harming healthy cells. Some new medicines, called targeted therapies, have exploited the overexpression of a specific protein or the mutation of a gene to develop a new medication to treat disease. One such example is the use of anti-EGF receptor medications to treat the subset of breast cancer tumors that have very high levels of the EGF protein. Undoubtedly, more targeted therapies will be developed as scientists learn more about how gene expression changes can cause cancer.

career CONNECTION

Clinical Trial Coordinator

A clinical trial coordinator is the person managing the proceedings of the clinical trial. This job includes coordinating patient schedules and appointments, maintaining detailed notes, building the database to track patients (especially for long-term follow-up studies), ensuring proper documentation has been acquired and accepted, and working with the nurses and doctors to facilitate the trial and publication of the results. A clinical trial coordinator may have a science background, like a nursing degree, or other certification. People who have worked in science labs or in clinical offices are also qualified to become a clinical trial coordinator. These jobs are generally in hospitals; however, some clinics and doctor’s offices also conduct clinical trials and may hire a coordinator.

KEY TERMS

3' UTR 3' untranslated region; region just downstream of the protein-coding region in an RNA molecule that is not translated

5' cap a methylated guanosine triphosphate (GTP) molecule that is attached to the 5' end of a messenger RNA to protect the end from degradation

5' UTR 5' untranslated region; region just upstream of the protein-coding region in an RNA molecule that is not translated

activator protein that binds to prokaryotic operators to increase transcription

catabolite activator protein (CAP) protein that complexes with cAMP to bind to the promoter sequences of operons which control sugar processing when glucose is not available

***cis*-acting element** transcription factor binding sites within the promoter that regulate the transcription of a gene adjacent to it

Dicer enzyme that chops the pre-miRNA into the mature form of the miRNA

DNA methylation epigenetic modification that leads to gene silencing; a process involving adding a methyl group to the DNA molecule

enhancer segment of DNA that is upstream, downstream, perhaps thousands of nucleotides away, or on another chromosome that influence the transcription of a specific gene

epigenetic heritable changes that do not involve changes in the DNA sequence

eukaryotic initiation factor-2 (eIF-2) protein that binds first to an mRNA to initiate translation

gene expression processes that control the turning on or turning off of a gene

guanine diphosphate (GDP) molecule that is left after the energy is used to start translation

guanine triphosphate (GTP) energy-providing molecule that binds to eIF-2 and is needed for translation

histone acetylation epigenetic modification that leads to gene silencing; a process involving adding or removing an acetyl functional group

inducible operon operon that can be activated or repressed depending on cellular needs and the surrounding environment

initiation complex protein complex containing eIF-2 that starts translation

***lac* operon** operon in prokaryotic cells that encodes genes required for processing and intake of lactose

large 60S ribosomal subunit second, larger ribosomal subunit that binds to the RNA to translate it into protein

microRNA (miRNA) small RNA molecules (approximately 21 nucleotides in length) that bind to RNA molecules to degrade them

myc oncogene that causes cancer in many cancer cells

negative regulator protein that prevents transcription

operator region of DNA outside of the promoter region that binds activators or repressors that control gene expression in prokaryotic cells

operon collection of genes involved in a pathway that are transcribed together as a single mRNA in prokaryotic cells

poly-A tail a series of adenine nucleotides that are attached to the 3' end of an mRNA to protect the end from